(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
13 June 2002 (13.06.2002)

PCT

(10) International Publication Number
WO 02/46430 A2

(51) International Patent Classification[7]: C12N 15/62, C07K 14/54, A61K 38/20

(21) International Application Number: PCT/IL01/01125

(22) International Filing Date: 5 December 2001 (05.12.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
140110 5 December 2000 (05.12.2000) IL

(71) Applicant *(for all designated States except US)*: APPLIED RESEARCH SYSTEMS ARS HOLDING N.V [NL/NL]; Pietermaai 15, Curacao (AN).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **AMITAI, Hagit** [IL/IL]; 4/30Paldi St., 76248 Rehovot (IL). **CHITLARU, Edith** [IL/IL]; 16/34 Hanassi Harishon St., 76302 Rehovot (IL).

(74) Agent: **EINAV, Henry**; Interpharm Laboratories Ltd., Science-based Industrial Park, Kiryat Weizmann, 76110 Ness Ziona (IL).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: IMPROVEMENT OF HOMOGENEITY AND SECRETION OF RECOMBINANT PROTEINS IN MAMMALIAN SYSTEMS

hGH-sp

5'hGH-IL-18BP fusion out primer: TAT AAGCTT ACC ATG GCT ACA GGC TCC CGG ACG T

IL-18BP hGH-sp

3' hGH-IL-18BP fusion-in primer: T GGT CTG CGA GAC AGG TGT GGC ACT GCC CTC TTG

hGH-sp IL-18BP

5'hGH-IL-18BP fusion-in primer: CAA GAG GGC AGT GCC ACA CCT GTC TCG CAG ACC A

Stop Stop IL-18BP

3'hGH-IL-18BP fusion-out primer: CG GGATCC CTA TTA ACC CTG CTG CTG TGG AC

(57) Abstract: The present invention relates to a method for improving homogeneity and/or secretion of a recombinant protein of interest expressed in mammalian cells by replacing the endogenous signal peptide sequence of the DNA encoding the protein of interest with that of human hGH. The invention also relates to DNA expression vectors containing the sequence encoding such proteins fused to the signal peptide sequence of the hGH and to cells harboring such vectors.

WO 02/46430 A2

Published:

— *without international search report and to be republished upon receipt of that report*

IMPROVEMENT OF HOMOGENEITY AND SECRETION OF RECOMBINANT PROTEINS IN MAMMALIAN SYSTEMS

FIELD OF THE INVENTION

The present invention relates to a method for improving homogeneity and/or secretion of a recombinant protein of interest expressed in mammalian cells by replacing the endogenous signal peptide sequence of the DNA encoding the protein of interest with that of human hGH. The invention also relates to DNA expression vectors containing the sequence encoding such proteins fused to the signal peptide sequence of the hGH and to cells harboring such vectors.

BACKGROUND OF THE INVENTION

Protein secretion is one of the most important issues of protein production in the field of biotechnology. This process is composed of the following steps: first, translocation across the endoplasmic reticulum (ER) membrane; second N-glycosylation and folding in the ER lumen; third, exit from the ER; fourth, modifications in the Golgi apparatus; and finally release from the secretory granules to the extracellular space (Sakaguchi 1997). Whether or not a protein is secreted from the cells mainly depends on whether it can be translocated across the membrane and whether it can be correctly folded in the ER lumen. Membrane translocation is obligatorily coupled in mammalian cells. After membrane translocation, the nascent peptides are released into the lumenal space and folded with the assistance of various chaperones and folding enzymes. Wrongly folded proteins are trapped within the ER and thus cannot proceed towards the secretory compartments. In biotechnological processes in which massive protein expression occurs, secretion can represent a bottleneck and limit the rate of expression.

Signal peptides or leader sequences, are located at the amino terminus of nascent polypeptides. They target proteins to the secretory pathway and are cleaved from the nascent chain once translocated in the reticulum endoplasmatic membrane.

The signal peptide consists of three regions: an amino-terminal polar region (N region), where positive charged aminoacid residues are frequently observed; a central hydrophobic region (H region) of more than 7-8 hydrophobic amino acid residues; and a carboxy-terminal region (C region) that includes the signal peptide cleavage site (Sakaguchi 1997). The eukaryotic H regions are dominated by Leu with some occurrence of Val, Ala, Phe and Ile. The cleavage of the signal peptide from the mature protein occurs at a specific site and the cleavage specificity resides in the last residue of the signal sequence (Nielsen et al. 1997). Close to the cleavage site –3 and –1 alanine is more predominant. This site confers processing specificity. No further specific patterns in the first few positions of the mature protein can be seen in eukaryotic organisms (Nielsen et al 1997). Therefore a "bad" signal peptide can promote more than one specific cleavage resulting in non-homogenous expression of the protein; i.e. the protein will be expressed with different N-terminal aminoacids.

Since many proteins are regulated under physiological conditions the use of natural regulatory signals for overexpression in mammalian systems is not desirable. For example, in such systems efficient promoters such as CMV and SV40 are used to control expression of recombinant proteins of interest. Similarly, the use of effective signal peptides such as SV40 and hGH poly A to overexpress recombinant secreted proteins instead of their endogenous counterparts would be advantageous.

The signal peptide of the human growth hormone (hGH) has been described to be effective in targeting the secretion of intracellular, membrane bound proteins and proteins secreted by different mechanisms than those governed by signal peptides.

For example, WO26562 describes the secretion of the intracellular protein icIL-1ra-II by fusion of the signal peptide of hGH to the sequence of the icIL-1ra-II. The invention relates to a process for the recombinant expression of a protein having the amino acid sequence of natural icIL-1ra-II in a recombinant cell expression system through use of a vector which is a fusion of the signal peptide of a human secretory protein, preferably the 26 amino acid signal peptide of hGH, fused in proper reading

frame with the DNA encoding icIL-1ra-II. The process comprises producing an expression vector containing DNA encoding icIL-1ra-II, either in the form of cDNA or genomic DNA, fused in proper reading frame with DNA encoding the selected signal peptide, preferably the 26 amino acid hGH signal peptide (Figure 1 SEQ ID NO:2). The expression vector is then inserted into an appropriate expression host, such as CHO cells. The transformed host cells are then cultured in a manner, which causes the expression vector to express its encoded protein, and the expressed and secreted icIL-1ra-II protein is then collected and purified from the culture medium.

Morris at al. (1999) describes the use of hGH signal peptide for the secretion of the protein CDL40L, which exists in nature predominantly as a membrane-anchored molecule. Several reports have shown that the soluble form of CD40L is biologically active (Fanslow et al. 1994, Hollenbaugh et al. 1992 and Mazzei et al. 1995). To use CD40L as a potential therapeutic, optimisation of soluble forms of this molecule have been developed. In this work, the activity of soluble forms of CD40L, and the activity of the soluble multimerized CD40L TNF homologous region, have been compared. The soluble forms of CD40L have been prepared by fusion of the entire extracellular domain of human CD40L or the CD40L region homologous to TNF sequence to the signal peptide sequence of hGH. The multimerized form of the CD40L has been prepared by fusion to an isoleucine zipper (IZ). The results showed that multimerization increases the activity of soluble CD40L.

Pecceu et al (1991) describes the use of the hGH signal peptide to express and secrete the mature form of IL-1β. In the body, after synthesis, proIL-1β remains primarily cytosolic until it is cleaved and transported out of the cells. Examination of the sequence reveals the absence of a classical N-terminal or internal hydrophobic signal peptide. Release of mature IL-1β appears to be linked to processing at the aspartic acid-alanine peptide cleavage by the converting enzyme (ICE) (Dinarello 1996). Although ICE is constitutively expressed in most cells, not all cells process proIL-1β and secrete mature IL-1β. Therefore secretion of mature IL-1β is cell dependent. Pecceu discloses the use a recombinant vector containing only the DNA encoding the mature form of IL-1β, without any signal peptide and a vector containing the DNA encoding the mature form of IL-1β

joined to hGH signal peptide. The results show that only 52% of the protein are secreted using the first construct, while using the construct with hGH signal peptide results in 97% secretion.

The first ATG codon for initiation of translation has to be identified by the transcriptional machinery. An ATG codon in a very weak context is not likely to be the start site for translation. The optimal context for initiation of translation in vertebral mRNAs is a G residue following the ATG codon (position +4 in the coding region) and a purine, preferable A, three nucleotides upstream (-3 in the noncoding region) this consensus sequence has been designated Kozak sequence (Kozak 1996, 1999). Messenger RNA in which the first ATG codon lacks the preferred nucleotides in both of these key flanking positions (a "bad " or non optimal Kozak sequence) have the special property of initiating translation at the first and second ATG codons, thereby producing two proteins from one RNA. The ATG in the initiation site of hGH signal peptide is followed by G (in position +3) required for obtaining an optimal Kozak sequence (Figures 1 and 2 SEQ ID NO:3), which ensures the start of translation at the first ATG site only and homogeneity of the product.

IL-18 binding protein (IL18-BP) was affinity purified from human urine using IL-18, sequenced and cloned. IL-18BP was found to abolish in vitro the activity of the pro-inflammatory cytokine IL-18. (Novick et al. 1999). The DNA encodes a signal peptide at its N- terminal portion. Part of the Kozak sequence encoded inside the signal peptide is not of the appropriate context.

IL-18BP leader	ATG	A
	+1	+4
Kozak Concensus	ATG	G
	+1	+4
Human growth hormone leader	ATG	G
	+1	+4

Therefore exists a need to provide a method or system allowing secretion of a protein of interest in general and allowing secretion of IL-18BP in particular as homogenous proteins and/or in large amounts in mammalian cells.

SUMMARY OF THE INVENTION

The invention provides an improved method for production of homogeneous recombinant protein of interest expressed in different mammalian systems and or effective secretion thereof comprising replacing the endogenous signal peptide sequence of the DNA encoding the protein of interest with that of hGH.

The invention also provides an expression vector for improving homogeneity and/or secretion of a recombinant protein of interest expressed in a mammalian system comprising the signal peptide sequence of the hGH joined to the DNA encoding the protein of interest.

In one aspect, the present invention provides said vector, which encodes the optimal Kozak sequence ensuring translation from one initiation codon only. Part of such a sequence is included in the coding for the hGH signal peptide, ensuring accurate cleavage of the signal peptide from the mature protein.

In one embodiment of the present invention the vector includes the gene encoding IL-18BP. Such a vector allows the production of homogeneous IL-18BP (starting with Thr-Pro-Val).

The invention also provides a protein of interest produced with the said vector and by the said method, such as IL-18BP.

In onother aspect, the invention provides cells capable of growing in serum free medium and able to produce at least 4 picogram IL-18B/cell/24 hours, preferably at least 11.8 picogram/cell/24 hours and most preferably about 12 picograms/cell/24 hours.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the genomic sequence encoding the hGH signal peptide and translated amino acid sequence.

Figure 2 shows the Kozak sequence present in the consensus and in the hGH signal peptide.

Figure 3 describes the primers used for the fusion of hGH signal peptide (without its intron) sequence with the IL-18BP sequence.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the production and secretion of recombinant proteins using the signal peptide sequence of the hGH instead of their natural signal peptide sequence. The invention also provides an expression vector containing the DNA encoding the protein of interest, either in the form of cDNA or genomic DNA, fused in proper reading frame with the DNA encoding the hGH signal peptide. The expression vector is then inserted into an appropriate expression host, i.e. mammalian cells. The transformed host cells are then cultured in a manner, which causes the expression vector to express its encoded protein, and the expressed and secreted protein is then isolated and purified. The expression of the protein of interest may be stable or transient.

While CHO cells are the preferred host cells, other mammalian cells may be used, such as COS cells, HEK293 cells etc. Those of ordinary skill in the art are well aware of the techniques of creating expression vectors, inserting them into expression systems and selecting clones, which express the desired protein, including amplification techniques.

As will be appreciated by those skilled in the art, the types of promoters used to control transcription of the recombinant proteins may be any of those, which are functional in the host cells. Examples of promoters functional in mammalian cells include the SV40 early promoter, adenovirus major late promoter, herpes simplex (HSV) thymidine kinase promoter, rous sarcoma (RSV) LTR promoter, human cytomegalovirus (CMV) immediate early promoter, mouse mammary tumor virus (MMTV) LTR promoter, interferon-β promoter, heat shock protein 70 (hsp 70) promoter, as well as many others well known in the art. These promoters may be either constitutive or regulatable. Constitutive promoters are preferred because an extra treatment step, such as temperature shift, addition of chemical agents or inducers, etc., is not required for expression from constitutive promoters. It has been shown in one embodiment that higher productivity can be obtained by controlling IL-18BP expression with the CMV promoter as compared to the SV40 promoter.

In mammalian cells, three elements define the core polyadenylation signal i.e. the highly conserved hexanucleotide AAUAAA found 10 to 30 nucleotides upstream of the cleavage site, a less highly conserved U-rich or GU-rich element located downstream of the cleavage site, and the cleavage site itself, which becomes the point of poly(A) addition and is thus generally referred to as the poly(A) site (Zhao et al. 1999). Additional sequences outside of this core recruit regulatory factors or maintain the core signal in an open and accessible structure. Different expression vectors for heterologous expression have been designed to contain efficient polyadenylation signals such as SV40 and hGH polyadenylation signals. In a preferred embodiment IL-18BP is produced using the human growth hormone polyadenylation signal.

In particular, the present invention relates to an expression vector which contains the IL-18BP DNA fused to the DNA encoding the signal peptide of hGH, and to host cells transfected with such an expression vector.

The DNA sequence of the hGH signal peptide used, may be either the genomic sequence including the intron (Figure 1 SEQ ID NO:1) or the cDNA sequence of the signal peptide excluding the intron.

In accordance with the present invention it is possible to express, IL-18BP and other secreted proteins homogeneously and effectively in a mammalian expression system using the signal peptide of hGH.

In addition, the invention relates to mammalian cells which express and efficiently secrete homogenous recombinant proteins using the hGH signal peptide.

In another aspect, the invention relates to cells which efficiently secrete homologous recombinant proteins, according to the invention, that are capable of being grown and produced in serum free medium (SFM). More specifically, in a preferred embodiment cells, according to the invention, were shown to produce between 4 and 11.8, and also about 12 picogram IL-18BP/cell/24 hours in both serum and serum free conditions.

The invention also relates to proteins of interest prepared according to the method herein described. In another aspect, the invention relates to pharmaceutical compositions comprising proteins of interest, such as IL-18BP produced in such an expression system, optionally together with a pharmaceutically acceptable excipient.

The invention will be now illustrated by the following non-limiting examples.

EXAMPLES

Example 1 establishment of the IL-18BP producing clone S10-21.

The DNA of the natural signal peptide of the IL-18BP protein encodes for a non-optimal Kozak sequence because the + 4 is A and not G therefore it was replaced by hGH signal peptide which exhibits an optimal Kozak sequence (Figure 2 SEQ ID NO:3). The DNA fragment encoding the hGH signal peptide fused to the IL-18BP protein was introduced in a mammalian expression vector under the control of the SV-40 promoter and polyadenylation signal.

For the preparation of the expression vector, hGH signal peptide (without its intron, see figure 1) was fused directly to the cDNA coding for the mature hIL-18BP protein (variant A, accession number AF110799 in the NCBI public databases) by PCR (Figure 3 describes the primers used).

The hGH signal peptide (without its intron) was amplified by PCR using pXGH5 (Selden et al. 1986) as a template and two primers, a) a primer containing sequences from the beginning of the hGHsp (5'hGH-IL-18BP fusion out primer SEQ ID NO:4) and b) a primer encoding sequences from the end of the hGHsp and sequences coding the first 19 nucleotides of the mature IL-18BP cDNA (3' hGH-IL-18BP fusion-in primer SEQ ID NO:5).

The cDNA coding for the mature IL-18BP was amplified by PCR using a plasmid encoding the IL-18BP cDNA, as a template and two primers, a) a primer coding overlapping sequences to the hGH signal peptide end and the beginning of the mature IL-18BP cDNA (the 5'hGH-IL-18BP fusion-in primer SEQ ID NO:6) and b) a primer containing the end of the IL-18BP cDNA sequence (the 3'hGH-IL-18BP fusion-out primer SEQ ID NO:7).

The fragments resulting from the above PCR amplification were fused in a third PCR, by annealing of the overlapping sequences present in both fragments and using two primers a) the primer 5'hGH-IL-18BP fusion out, and b) the primer 3'hGH-IL-18BP fusion-out primers.

The resulting PCR DNA fragment was cloned into the mammalian expression vector pSVE3 (Hartman et al. 1982) encoding the commonly used regulatory signals, the SV40 promoter and SV40 polyA signal (the 5' and 3' fusion out primers contained also specific restriction sites sequences needed for cloning).

The constructed plasmid (PSIL18BP) was used for transfecting CHO (DHFR-) cells together with a plasmid containing the mouse DHFR gene as a selective marker.

Individual isolates were isolated in selective medium and assayed for IL-18BP production by an ELISA assay.

Several rounds of gene amplification with increasing MTX concentrations were carried out. After amplification, clones were isolated by limiting dilution. After subcloning the selected clone S10-21 showed a specific and stable productivity of 1 picogram/cell/24 hours.

Example 2 Establishment of the IL-18BP producing clone 22C2-11.

The DNA of the natural signal peptide of the IL-18BP protein encodes for a non-optimal Kozak sequence because the + 4 is A and not G therefore it was replaced by hGH signal peptide which exhibits an optimal Kozak sequence (Figure 2 SEQ ID NO:3).). The DNA fragment encoding the hGH signal peptide fused to the IL-18BP protein was introduced in a mammalian expression vector under the control of the CMV promoter and the human growth hormone polyadenylation signal.

For the preparation of the expression vector, the DNA encoding the hGH signal peptide (without its intron see figure 1) was fused directly to the cDNA coding for the mature hIL-18BP protein (variant A accession number AF110799) by PCR using the expression vector generated in example 1 (PSIL18BP) as a template and two primers, a) the forward primer ACGCGTTCGACGCCACCATGGCTCCCGGACG (SEQ ID NO:8) comprising a SalI restriction site, and the first 21 bases encoding the cDNA hGH signal peptide and b) the reverse primer CGGGATCCTCATTAACCCTGCTGCTGTGG (SEQ ID NO:9) comprising the last 18 bases of IL-18BP, two stop codons and a Bam HI restriction site.

The resulting PCR DNA fragment was inserted into a mammalian expression vector, using known molecular genetic manipulations, wherein the mammalian vector comprise the commonly used regulatory signals to be used to express IL-18BP: the CMV promoter and hGH polyA signal (Ausubel *et al.*, Current Protocols in Molecular Biology, Greene Publications *and Wiley Interscience, New York, NY*, 1987-1995; Sambrook *et al.*,

Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989).

The constructed plasmid (pCMV-IL18bp2) was used for transfecting CHO (DHFR-) cells together with a plasmid containing the mouse DHFR gene as a selective marker.

Individual isolates were isolated in selective medium and assayed for IL-18BP production by an ELISA assay.

Several rounds of gene amplification with increasing MTX concentrations were carried out (up to 1000 nM) on selected high producer isolates. Following amplification the MTX was removed and high producer clones were isolated by limiting dilution and screening the activity. After cloning the selected clone 22C2-11 showed high specific productivity of 4 picogram/cell/24 hours and stability in serum at 37°C. The 22C2-11 clone was found to grow and produce IL-18BP also under serum free conditions. The productivity of the clone 22C2-11 was tested at lower temperatures. At 32-33°C, both in serum and serum free conditions, the productivity was found to increase to 11.8 picogram IL-18BP/cell/24 hours.

These results indicate that using a construct controlling expression from the CMV promoter and hGH ployA signal allowed increased IL-18BP expression as compared to the expression levels controlled from the SV40 promoter and SV40 poly A signal (example 1).

Example 3 Purification and N-terminal analysis of the IL-18BP produced.

The IL-18BP in the supernatant of all the producing cells (S10-21 and 22C2-11 examples 1 and 2 respectively) was purified by immunoaffinity chromatography. N-terminal analysis of the IL18-BP expressed with the signal peptide of hGH, revealed only the correct species of IL-18B with the following N-terminal amino acid sequence T P V S Q T T T A A T A S V R (SEQ ID NO:10). These results show that IL-18BP is

homogeneously produced, from different expression vectors, by using the hGH signal peptide in which, in contrast to the IL-18BP natural signal peptide, the Kozak signal is of optimal context.

REFERENCES

Dinarello, C.A. (1996) "Biologic Basis for Interleukin-1 in Disease."Blood, 87, 2095-147.

Fanslow, W.C., Srinivasan, S., Paxton, R., Gibson, M.G., Spriggs, M.K., Armitage, R.J. (1994) "Structural characteristics of CD40 ligand that determine biological function." Semin Immunology, 5, 267-78.

Hartman, J.R., Nayak, D.P. and Fareed, G.C. (1982) "Human Influenza virus hemagglutinin is expressed in monkey cells using simian virus 40 vectors."Proc.Natl.Acad.Sci.USA, 79, 233-7.

Hollenbaugh, D., Grosmaire, L.S., Kullas, C.D., Chalupny, N.J., Braesch-Andersen, S., Noelle, R.J., Stamenkovic, I., Ledbetter, J.A., and Aruffo, A. (1992) "The human T cell antigen gp39, a member of the TNF gene family, is a ligand for the CD40 receptor: expression of a soluble form of gp39 with B cell co-stimulatory activity."

EMBO J. 11, 4313-21.

Kozak, M. (1996)"Interpreting cDNA sequences: some insights from studies on translation." Mammalian Genome, 7, 563-74.

Kozak, M. (1999) "Initiation of translation in prokaryotes and eukaryotes." Gene, 234, 187-208.

Mazzei, G.J., Edgerton, M.D., Losberger, C., Lecoanet-Henchoz, S., Graber, P., Durandy, A., Gauchat, J.F., Bernard, A., Allet, B. and Bonnefoy, J.Y. (1995) "Recombinant Soluble Trimeric CD40 Ligand Is Biologically Active."

J. Biol. Chem. 270, 7025-8.

Morris, A., Remmele, Jr., Klinke, R., Macduff, B.M., Fanslow, W.C. and Armitage, R.J. (1999) "Incorporation of an Isoleucine Zipper Motif Enhances the Biological Avtivity of Soluble CD40L (CD154)." The journal of Biological Chemistry, 474, 418-23.

Nielsen, H., Engelbrecht, J., Brunak, S. and von Heijne, G. (1997) "Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites." Protein Engineering, 10, 1-6.

Novick, D., Kim,S.H., Fantuzzi, G., Reznikov, L.L, Dinarello, C.A. and Rubinstein, M.(1999) "Interleukin-18 Binding Protein: A Novel Modulator of the Th1 Cytokine Response." Immunity 10, 127-136.

Pecceu, F., Dousset, P., Shire, D., Cavrois, E., Marchese, E., Ferrara, P., Kaghad, M., Dumont, X. and Lupker, J.(1991) "human interleukin 1b fused to the human growth hormone signal peptide is N-glycosilated and secretede by chinese hamster ovary cells." Gene, 97, 253-8.

Sakaguchi, M. (1997) "Eukaryotic protein secretion." Current Opinion in Biotechnology, 8, 595-601.

Selden, R.F., Howie, K.B., Rowe, M.E., Goodman, H.M. and Moore, D.D. (1986) "Human growth hormone as a reporter gene in regulation studies employing transient gene expression." Mol Cell Biol 6, 3173-9.

Zhao, J., Hyman, L., and Moore, C. (1999) "Formation of mRNA 3' ends in eukaryotes: mechanism, regulation and interrelationships with other steps in mRNA synthesis." Microbiology and Molecular Biology Reviews 63, 405-445.

CLAIMS

1. A method for improving homogeneity and/or secretion of a recombinant protein of interest expressed in a mammalian system comprising replacing the endogenous signal peptide sequence of the DNA encoding the protein of interest with that of hGH.
2. A method according to claim 1, wherein the protein of interst IL-18BP.
3. A method according to claim 2, wherein the mammalian system comprises CHO cells.
4. A method according to claim 3, wherein the CHO cells are cultured in medium supplemented with serum.
5. A method according to claim 3, wherein the CHO cells are cultured under serum free conditions.
6. An expression vector for improving homogeneity and/or secretion of a recombinant protein of interest expressed in a mammalian system comprising the signal peptide sequence of hGH and the DNA encoding the protein of interest.
7. An expression vector according to claim 6, which directs expression of a homogenous protein.
8. An expression vector according to claim 6, which directs efficient expression of the protein of interest.
9. An expression vector according to claim 6, which directs efficient translation of the protein of interest.
10. An expression vector according to claim 6, in which the signal peptide encodes an optimal Kozak sequence.

11. An expression vector according to claim 6, which directs efficient secretion of the protein of interest.

12. An expression vector according to claim 6, which allows constitutive expression of the protein of interest.

13. An expression vector according to claim 6, wherein the expression of the gene encoding the protein of interest is regulated by the CMV promoter.

14. An expression vector according to claim 6, wherein the expression of the gene encoding the protein of interest is regulated by the polyadenylation signal of the human growth hormone.

15. An expression vector in accordance with anyone of claims 6 to 14, wherein the protein of interest is IL-18BP.

16. A cell harbouring a vector according to anyone of claims 6 to 15.

17. A cell according to claim 16, producing about 12 picogram IL-18BP/cell/24 hours.

18. A cell according to claim 16, producing 11.8 picogram IL-18BP/cell/24 hours.

19. A cell according to claim 16, producing at least 4 picogram IL-18BP/cell/24 hours.

20. A cell according to claim 16, producing at least 11.8 picogram IL-18BP/cell/24 hours.

21. A cell according to claim 16, capable of growing in serum free medium.

22. A cell according to claim 21, producing at least 4 picogram IL-18 BP/cell/24 hours.

23. A cell according to claim 21, producing about 12 picogram IL-18 BP/cell/24 hours.

24. A cell according to claim 21, producing 11.8 picogram IL-18BP/cell/24 hours.

25. A cell according to claim 21, producing at least 11.8 picogram IL-18BP/cell/24 hours.

26. A protein obtainable by the method of claim 1.

27. A protein according to claim 26, being IL-18BP.

28. A pharmaceutical composition comprising a protein according to claims 26 or 27.

Figure 1

Exon 1

ATG GCT ACA G

Met Ala Thr G

Intron 1

GTAAGCGCCC CTAAAATCCC TTTGGGCACA ATGTGTCCTG AGGGGAGAGG CAGCGACCTG
TAGATGGGAC GGGGGCACTA ACCCTCAGGT TTGGGGCTTC TGAATGTGAG TATCGCCATG
TAAGCCCAGT ATTTGGCCAA TCTCAGAAAG CTCCTGGTCC CTGGAGGGAT GGAGAGAGAA
AAACAAACAG CTCCTGGAGC AGGGAGAGTG CTGGCCTCTT GCTCTCCGGC TCCCTCTGTT
GCCCTCTGGT TTCTCCCCAG

Exon 2

GC TCC CGG ACG TCC CTG CTC CTG GCT TTT GGC CTG CTC TGC

ly Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu Leu Cys

signal peptide cleavage site

CTG CCC TGG CTT CAA GAG GGC AGT GCC TTC CCA ACC ATT CCC TTA TCC AGG

Leu Pro Trp Leu Gln Glu Gly Ser Ala Phe Pro Thr Ile Pro Leu Ser Arg

Figure 2

Kozak Concensus	ATG G +1 +4	
Human growth hormone leader	ATG G +1 +4	

Figure 3

			hGH-sp
5'hGH-IL-18BP fusion out primer:	TAT AAGCTT ACC ATG GCT ACA GGC TCC CGG ACG T		
	IL-18BP	hGH-sp	
3' hGH-IL-18BP fusion-in primer:	T GGT CTG CGA GAC AGG TGT GGC ACT GCC CTC TTG		
	hGH-sp	IL-18BP	
5'hGH-IL-18BP fusion-in primer:	CAA GAG GGC AGT GCC ACA CCT GTC TCG CAG ACC A		
	Stop Stop IL-18BP		
3'hGH-IL-18BP fusion-out primer:	CG GGATCC CTA TTA ACC CTG CTG CTG TGG AC		

SEQUENCE LISTING

<110> APPLIED RESEARCH SYSTEMS ARS HOLDING N.V

<120> IMPROVEMENT OF HOMOGENEITY AND SECRETION OF RECOMBINANT PROTEINS IN MAMMALIAN SYSTEMS

<130> IL/470

<150> IL/140110

<151> 2000-12-05

<160> 10

<170> PatentIn version 3.1

<210> 1

<211> 338

<212> DNA

<213> Homo sapiens

<400> 1

```
atggctacag gtaagcgccc ctaaaatccc tttgggcaca atgtgtcctg aggggagagg      60

cagcgacctg tagatgggac gggggcacta accctcaggt ttggggcttc tgaatgtgag     120

tatcgccatg taagcccagt atttggccaa tctcagaaag ctcctggtcc ctggagggat     180

ggagagagaa aaacaaacag ctcctggagc agggagagtg ctggcctctt gctctccggc     240

tccctctgtt gccctctggt ttctccccag gctcccggac gtccctgctc ctggcttttg     300

gcctgctctg cctgccctgg cttcaagagg gcagtgcc                             338
```

<210> 2

<211> 26

<212> PRT

<213> Homo sapiens

<400> 2

Met Ala Thr Gly Ser Arg Thr Ser Leu Leu Leu Ala Phe Gly Leu Leu

1 5 10 15

Cys Leu Pro Trp Leu Gln Glu Gly Ser Ala
20 25

<210> 3

<211> 4

<212> DNA

<213> Homo sapiens

<400> 3
atgg 4

<210> 4

<211> 34

<212> DNA

<213> Homo sapiens

<400> 4
tataagctta ccatggctac aggctcccgg acgt 34

<210> 5

<211> 34

<212> DNA

<213> Homo sapiens

<400> 5
tggtctgcga gacaggtgtg gcactgccct cttg 34

<210> 6

<211> 34

<212> DNA

<213> Homo sapiens

<400> 6
caagagggca gtgccacacc tgtctcgcag acca 34

<210> 7

<211> 31

<212> DNA

<213> Homo sapiens

<400> 7
cgggatccct attaaccctg ctgctgtgga c 31

<210> 8

<211> 31

<212> DNA

<213> Homo sapiens

<400> 8
acgcgttcga cgccaccatg gctcccggac g 31

<210> 9

<211> 29

<212> DNA

<213> Homo sapiens

<400> 9
cgggatcctc attaaccctg ctgctgtgg 29

<210> 10

<211> 15

<212> PRT

<213> Homo sapiens

<400> 10

Thr Pro Val Ser Gln Thr Thr Thr Ala Ala Thr Ala Ser Val Arg
1 5 10 15